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Naval Medical Research Institute

Bethesda, MD 20814-5055

NMRI 88-94

December 1988



AD-A215 986

ARGININE VASOPRESSIN LOWERS PULMONARY ARTERY
PRESSURE IN HYPOXIC RATS BY RELEASING
ATRIAL NATRIURETIC PEPTIDE

H. Jin

Y-F. Chen

R-H. Yang

T. M. McKenna

R. M. Jackson

S. Opari

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Naval Medical Research
and Development Command
Bethesda, Maryland 20814-5044

Department of the Navy
Naval Medical Command
Washington, D.C. 20372-5210

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a REPORT SECURITY CLASSIFICATION UNCLASSIFIED			1b RESTRICTIVE MARKINGS	
2a SECURITY CLASSIFICATION AUTHORITY			3 DISTRIBUTION AVAILABILITY OF REPORT	
2b DECLASSIFICATION/DOWNGRADING SCHEDULE				
4 PERFORMING ORGANIZATION REPORT NUMBER(S) NMRI 88-94			5 MONITORING ORGANIZATION REPORT NUMBER(S)	
6a NAME OF PERFORMING ORGANIZATION Naval Medical Research Institute		6b OFFICE SYMBOL (If applicable)	7a NAME OF MONITORING ORGANIZATION Naval Medical Command	
6c ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5055			7b ADDRESS (City, State, and ZIP Code) Department of the Navy Washington, D. C. 20372-5120	
8a NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research & Development Command		8b OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5044			10 SOURCE OF FUNDING NUMBERS	
			PROGRAM ELEMENT NO	
			PROJECT NO	
			TASK NO	
			WORK UNIT ACCESSION NO	
			62233N	
			MM33C20	
			MM33C30.01	
			DN246558	
11 TITLE (Include Security Classification) ARGININE VASOPRESSIN LOWERS PULMONARY ARTERY PRESSURE IN HYPOXIC RATS BY RELEASING ATRIAL NATRIURETIC PEPTIDE				
12 PERSONAL AUTHOR(S) Hongkui Jin, Yiu-Fai Chen, Ren-Hui Yang, Thomas M. McKenna, Robert M. Jackson, Suzanne Opa				
13a TYPE OF REPORT Technical Report		13b TIME COVERED FROM _____ TO _____		14 DATE OF REPORT (Year, Month, Day) 06 Dec 88
15 PAGE COUNT 36				
16 SUPPLEMENTARY NOTATION				
17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	Vascular, Hypoxia, Vasopressin, Receptor	
19 ABSTRACT (Continue on reverse if necessary and identify by block number) We previously demonstrated that arginine vasopressin (AVP) lowers pulmonary artery pressure in rats with hypoxic pulmonary hypertension by activation of the V ₁ receptor. The pulmonary depressor effect of AVP in hypoxia adapted rats is not due to its effect on cardiac output. The current study tested two alternative hypotheses: that AVP lowers pulmonary artery pressure in the hypoxia adapted lung by 1) dilating pulmonary vasculature directly, or 2) releasing atrial natriuretic peptide (ANP) from the heart. The first hypothesis was tested by injecting AVP into the pulmonary arteries of isolated, buffer perfused lungs and monitoring pulmonary artery pressure, and by exposing precontracted pulmonary artery rings to graded doses of AVP and monitoring the tension generated. AVP caused minimal vasodilation in perfused lungs and only a small vasodilator effect in pulmonary artery rings. The second hypothesis was tested by injecting AVP (160 ng/kg) or vehicle intravenously in conscious hypoxia adapted (4 weeks) or air control rats and				
20 DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21 ABSTRACT SECURITY CLASSIFICATION	
22a NAME OF RESPONSIBLE INDIVIDUAL Regina E. Hunt, Command Editor			22b TELEPHONE (Include Area Code) (202) 2950198	
			22c OFFICE SYMBOL ISB/ADMIN/NMRI	

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measuring ANP in arterial blood and atria, and by testing pretreatment with the V_1 receptor antagonist $d(CH_2)_5$ Tyr(Me)AVP (130 μ g/kg) on the AVP-induced increase in plasma ANP. AVP produced a 7-fold increase in plasma ANP (209 ± 33 to 1346 ± 233 pg/ml; $p < 0.05$) in hypoxia adapted rats and a 5-fold increase in ANP (122 ± 22 to 573 ± 174 pg/ml; $p < 0.05$) in air controls. ANP release was abolished by pretreatment of both groups with $d(CH_2)_5$ Tyr(Me)AVP. The AVP induced ANP release came mainly from left atrium. These data strongly suggest that the pulmonary depressor effects of AVP in hypoxia adapted rats is due to augmented V_1 receptor-induced release of ANP from left atrium.

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ACKNOWLEDGEMENTS

A portion of this work was performed under Naval Medical Research and Development Command Work Unit No. MM33C30.01.1001. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. The excellent technical assistance of David Reusch and John Lueders and editorial assistance of Charlane Crouse are gratefully acknowledged. We would also like to thank Wyeth Pharmaceuticals for providing the anti-ANP antibody. The experiments reported herein were conducted according to the principles set forth in the "Guide for Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHHS, Pub. No. (NIH)86-23. This work has also been supported by grants from NIH/NHLBI HL-35051 and HL-22544; and the National Dairy Board, administered in cooperation with the National Dairy Council.

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INTRODUCTION

Previous studies (15,17) from our laboratory have demonstrated that arginine vasopressin (AVP) lowers pulmonary artery pressure in rats with hypoxic pulmonary hypertension. The pulmonary depressor effect of AVP in hypoxia-adapted rats was dose dependent and appeared to be mediated by activation of the V_1 receptor. In contrast, AVP had little effect on pulmonary artery pressure in air control rats. AVP in doses sufficient to lower pulmonary artery pressure elicited a systemic pressor response in hypoxia-adapted rats that was significantly blunted compared to air controls. To test the hypothesis that the pulmonary depressor action of AVP is due to the effects of the peptide on the heart and systemic vasculature, we measured cardiac output before and after AVP administration in hypoxia-adapted and air control rats (17). The AVP did not cause a greater reduction in cardiac output of hypoxia-adapted rats compared to air controls. Calculated pulmonary vascular resistance fell slightly following AVP administration in hypoxia-adapted rats, but increased in air controls. The hemodynamic data indicate that the AVP-induced fall in cardiac output fails to account for the decrease in pulmonary artery pressure in the hypoxic group.

In the current study, we examined the effects of AVP on the pulmonary vasculature of the hypoxia-adapted and air control rats in two preparations: isolated, non-blood perfused lungs and pulmonary artery rings. AVP had little vasodilator effect in perfused lungs, and only a small vasodilator effect in pulmonary artery rings. Further, since previous studies have shown that AVP induces the release of atrial natriuretic peptide (ANP) from isolated atria

(35) and increases circulating levels of ANP in intact rats (19), we then tested the hypothesis that AVP dilates the pulmonary vasculature in rats with hypoxic pulmonary hypertension by releasing ANP.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing approximately 220g were maintained in either 10% O_2 (hypoxia) or room air (normoxic controls) for periods of 28 days as previously described (14). Rats were exposed to hypoxia in a 330 liter plexiglas glove box (Manostat, Brooklyn, NY). Hypoxic exposures (range $10.0 \pm 0.5\%$ O_2), were accomplished by adding N_2 (Southern Welding, Birmingham, AL) to the chamber intermittently from a liquid N_2 reservoir, the gas outflow of which was regulated by a solenoid valve controlled by the recorder output of an S3-A O_2 analyzer (Applied Electrochemistry, Sunnyvale, CA) through a control circuit (model 371-K, LFE, Clinton, MA). A baralyme (Allied Health Care Products, St. Louis, MO) CO_2 scrubber kept the $[CO_2]$ at $<0.2\%$. Relative humidity within the chamber was kept at $<70\%$ with anhydrous $CaSO_4$. Boric acid was used to keep NH_3 levels within the chamber at a minimum. Animals were permitted to have standard laboratory chow and tap water ad libitum. Daily animal maintenance was carried out without interruption of the exposures. Control animals were caged similarly and were exposed to filtered room air for identical periods.

In the initial experiment, the direct effect of AVP on pulmonary arterial pressure in isolated perfused lungs was determined. Eight hypoxic and 8 normoxic control rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.) after 4 weeks of exposure to hypoxia or room air. A tracheostomy was performed, and a 15 gauge Luer stub was secured in the trachea. The lungs were ventilated with a Harvard rodent respirator (tidal volume, 1 ml/100 g body wt; rate, 60 min^{-1} ; end-expiratory pressure, 2.5 cm H_2O) using a 5% CO_2 -20% O_2 -75% N_2 gas mixture (Southern Welding, Birmingham, AL). The thoracic and abdominal cavities were opened. The rats were then killed by severing the

abdominal aorta and vena cava. A 16 gauge bulb-tipped cannula was inserted into the main pulmonary artery. The left atrium was transacted. The lungs were perfused free of blood using a peristaltic pump (2 ml/min) from a separate reservoir that contained perfusate at 37°C. When cleared of blood, the lungs were removed from the thorax and transferred to the perfusion chamber, where they were perfused with a similar peristaltic pump (Harvard Apparatus, South Natick, MA) at a flow rate of 8-12 ml/min in order to maintain MPAP at levels similar to those observed in vivo. The perfusate consisted of Krebs-Ringer bicarbonate buffer containing 3% fatty acid-free albumin (Bovuminar, Armour Pharmaceutical, Tarrytown, NY) and 5 mM glucose (pH 7.4; 37°C) (37). Perfusate was continuously equilibrated with ventilator gas in an aerator, and perfusate pH was monitored throughout the experiment with a flow-through pH cuvette. Pulmonary artery and airway pressures were monitored and recorded continuously, because they are reliable indicators of edema formation in this preparation (9). After a 10 min equilibration period, 50 ng (in 50 μ l saline). AVP ([Arg⁸]-vasopressin, Sigma Chemical Co., St. Louis, MO) (a dose previously shown to reduce MPAP by approximately 24% of the pretreatment level in intact conscious hypoxia-adapted rats). 50 μ l saline, or 12.5 mg hydralazine (Sigma Chemical Co., St. Louis, MO) were injected into the pulmonary artery cannula in random order. The interval between injections was 10-15 minutes. Maximal changes in MPAP after injection were recorded. Each pair of lungs received a total of 3 injections, and the total duration of each perfusion was 40-45 minutes. Lung wet weight-to-dry weight ratios (by oven drying at 80°C for 72 hr) were obtained after each perfusion period to monitor weight gain of the preparation.

In a second protocol, the influence of AVP on the contractile performance of thoracic aorta and pulmonary artery tissue isolated from rats adapted to hypoxia for 4 weeks or air controls was examined. The rats were killed by decapitation and the thoracic aorta and pulmonary artery were immediately excised. The adventitia was removed from both vessels and the attached remnant of the thoracic aorta was removed from the pulmonary artery with the aid of a binocular dissecting microscope. The pulmonary artery was cut into 1 ring approximately 2 mm in length. The thoracic aorta was sectioned into 1 ring 3.5 mm in length. De-endothelialized tissue was prepared from the remaining portion of the aorta, prior to sectioning into an additional ring, by perfusion of the isolated aorta with 2 mg/ml sodium deoxycholate in Krebs-Ringer Bicarbonate buffer (KRB) for 20 sec, followed by a 5 min wash with KRB (millimolar composition: NaCl 118, KCl 4.7, CaCl₂ 1.3, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25.0, glucose 11.7) at pH 7.4 while bubbled with 95% O₂-5% CO₂.

Each ring was mounted between 2 stainless steel hooks in a 10 ml jacketed organ bath and maintained in KRB at 37°C and continually gassed. One hook was stationary and the other was attached to an isometric force transducer (Kulite Semiconductor BG-10). Ring tension was recorded on a Gould Brush 2400 chart recorder. All rings were subjected to a conditioning protocol that included: 1) equilibration to a resting tension of 2.5 g for 30-45 min (preliminary experiments showed this to be the optimal length-tension relationship for ring contraction,) 2) application of norepinephrine (NE, 3×10^{-7} M) (norepinephrine bitartrate, Sigma Chemical Co., St. Louis, MO) to the organ bath with acetylcholine (ACh, 10^{-5} M) (acetylcholine chloride, Calbiochem Behringer, La Jolla, CA) added at maximal tension to confirm the functional presence or absence of the endothelium (11) and 3) application of an

additional dose of NE (3×10^{-7} M) for 20 min to assure that the rings could sustain a stable tension for this time period. The rings were flushed with KRB between each conditioning treatment until a stable baseline tension was reattained.

The influence of adaptation to hypoxia on thoracic aorta contractile response to AVP was examined by subjecting conditioned endothelium-intact or de-endothelialized aortic rings isolated from hypoxia-adapted and control rats to cumulative doses of AVP from 10^{-10} to 10^{-6} M. Vascular tissue contraction can be characterized by two distinct responses during exposure to an agonist: 1) sensitivity to the agonist and 2) development of tension. Therefore, both sensitivity to AVP and measured tension were taken into account by integrating the tension developed by aortic rings in response to the cumulative doses of AVP; i.e., mg tension/ng tissue versus $\ln [\text{AVP}]$ M. Integrated dose responses for aortic ring contractile responses to AVP were calculated over the entire dose range of 10^{-10} to 10^{-6} M. In addition, because large doses of AVP actually diminished vascular contraction in some preparations, integrated dose responses were also calculated over a subset of the dose range (10^{-10} to 3×10^{-8} M) in which all preparations displayed a maximal contraction. EC_{50} values (EC_{50} = concentration of AVP causing a half maximal contraction) were calculated by linear regression after logit-log transformation of dose responses.

The influence of adaptation to hypoxia on pulmonary artery relaxation to AVP was measured in conditioned pulmonary artery rings isolated from hypoxia-adapted and control rats. The rings were pre-contracted by 3×10^{-7} M NE and, after maximal contraction was attained, relaxation of the pulmonary vessels to AVP was induced by subjecting the rings to cumulative doses of AVP from 10^{-10}

to 10^{-8} M. Each sequential dose of AVP was applied after maximal relaxation had occurred in response to the preceding dose. All rings were removed from the organ baths, blotted and weighed after completion of the experiments.

In a third set of experiments, effects of AVP on plasma and atrial ANP levels were assessed in hypoxia adapted and air control rats. Polyethylene catheters (PE-10 fused to PE-50) filled with heparin-saline solution (50 U/ml) were implanted in the femoral artery and vein for blood sampling and drug administration respectively under ether anesthesia after 25 days of hypoxic or normoxic exposure. Following catheter implantation, all rats were housed individually, and the hypoxia adapted rats were returned to the hypoxic chamber. Two days after implantation, tubing was connected with the femoral arterial catheter for blood sampling. At least 1 hr was allowed to pass before 1.0 ml of blood was collected from conscious, unrestrained, resting animals 5 min after i.v. injection of AVP (160 ng/kg) or saline vehicle for ANP determination. The blood withdrawn was immediately replaced with an equal volume of 0.9% saline. Blood was placed in iced tubes containing 1.5 mg EDTA and 1 trypsin-inhibitor unit of aprotinin. Rats were then decapitated, and left and right atria were removed quickly. Plasma was separated by centrifugation. Plasma and atrial samples were stored at -80°C until radioimmunoassay (RIA) for ANP.

In a separate group of hypoxia-adapted and air control rats, the effects of AVP on MSAP and MPAP were examined. After 25 days of exposure to hypoxia or room air, catheters were implanted in the femoral artery and vein as indicated above. The pulmonary artery was catheterized in situ via the right jugular vein by a modification of the closed chest technique of Rabinovitch et al. (29), as described in our previous reports (16,17). Two days after the

catheterization, MSAP and MPAP were recorded in conscious rats, through the pulmonary and femoral arterial catheter using a Model CP-01 pressure transducer (Century Technology Company, Inglewood, CA) coupled to a Grass Model 7 polygraph (Grass Instrument Co., Quincy, MA). After stable MSAP and MPAP were obtained, AVP (160 ng/kg) or saline vehicle were injected intravenously and peak changes in MSAP and MPAP were recorded.

ANP content of plasma and atria was measured by a modification of the RIA of Tanaka et al. (38), Eskay et al. (8) and Nakao et al. (24). Plasma for ANP determination was extracted with Sep-Pak C-18 cartridges (Waters Associates, Milford, MA) by the method of Eskay et al. (8). Extracts were dried under vacuum and reconstituted in RIA buffer (see below). Tissue samples were prepared by a modification of the methods of Tanaka et al. (38) and Nakao et al. (24). Briefly, left and right atria were weighed and homogenized in 2 ml of 1 M acetic acid containing 20 mM HCl. The homogenate was heated in a boiling water bath for 10 min and centrifuged at 25,000 x g for 30 min at 4°C. The supernatant was lyophilized overnight and reconstituted in RIA buffer (see below).

Rat 8-33 ANP (Peninsula Labs, Belmont, CA) was used as the reference standard. Rabbit anti-rat alpha AVP antiserum was generously donated by Wyeth Laboratories. During the assay, 10 ul of standard (2-250 pg) or sample were incubated for 48 hrs at 4°C with 100 ul (8,000 cpm) of ^{125}I labeled rat ANP (DuPont/NEN Research Products, Boston, MA), 100 ul of ANP antiserum (twice the dilution recommended by the manufacturer) and 200 ul RIA buffer (50 mM potassium phosphate buffer, pH 7.4, containing 0.1% bovine serum albumin, 0.01% NaN_3 , 0.1% Triton X-100, 50 uM phenylmethylsulfonyl fluoride, 50 mM NaCl and 0.0005% aprotinin). Separation of bound from free tracer was done by

adding 750 μ l of 20% polyethylene glycol-8000 and 75 μ l of 1.5% bovine gamma globulin to each assay tube and centrifuging for 1 hr at 2,200g (18). Recovery of ANP from plasma, as assessed by addition of unlabeled 8-33 ANP to normal rat plasma, was $91 \pm 4\%$. Non-specific binding of the tracer was 3%. The sensitivity of the ANP-RIA was 3.3 pg/assay tube, with 50% displacement at 33 pg/assay tube.

The final experiment tested the functional significance of AVP-induced ANP release in mediating the pulmonary artery pressure lowering effect of AVP in hypoxia adapted rats. We had previously shown that the V_1 receptor antagonist $d(CH_2)_5$ Tyr(Me)AVP administered in a dose of 130 μ g/kg, i.v., inhibits completely the AVP-induced pulmonary depressor and systemic pressor effects in hypoxia adapted rats (15). We reasoned that, if the pulmonary depressor effect of AVP is secondary to ANP release, the V_1 receptor antagonist should also prevent ANP release in this setting. Accordingly, the effect of the V_1 receptor antagonist on the AVP-induced increase in plasma ANP was determined. The experiment was performed 2 days after catheterization of the femoral artery and vein as described above. AVP (160 ng/kg), was injected i.v. 5 min after $d(CH_2)_5$ Tyr(Me)AVP (130 μ g/kg), into hypoxia adapted and air control rats in the conscious, resting state. Five min later, 1 ml of blood was collected for ANP measurement as previously described.

Statistical Analysis. Results are expressed as mean \pm SEM. Comparisons of paired and independent mean treatment effects were by the appropriate Student's t-test in the first and second experiments. Comparisons of relaxation responses, expressed as a percentage of initial contraction, were by Mann-Whitney U-test (36). In the third experiment, results were analyzed by 2 way analysis of variance; significant differences were then subjected to Newman-Keuls post-hoc analysis.

RESULTS

Rats kept in hypoxia for 4 weeks had reduced body weights compared to air controls (Table 1). Baseline MPAP in isolated perfused lungs from hypoxia adapted rats was significantly higher ($p < 0.01$) than in those from air controls (Table 1). The wet weight-to-dry weight ratios of perfused lungs were between 6.0 and 6.5 in both hypoxic and normoxic groups, indicating that significant alveolar edema did not develop in the course of in vitro perfusion. Bolus injection of 50 ng AVP into the pulmonary cannula produced only a slight decrease in MPAP in isolated perfused lungs from both hypoxic and normoxic rats (Figure 1). Saline vehicle did not alter MPAP in either group. In contrast, hydralazine (12.5 mg) injection through the pulmonary cannula caused a significant decrease in MPAP in both groups. The pulmonary depressor response to hydralazine was not significantly different in hypoxia adapted rats from air controls.

Thoracic aorta and pulmonary artery rings, pre-contracted by NE during the conditioning protocol, exhibited robust endothelium-dependent relaxation in response to Ach (at least 40% by both hypoxia-adapted and control rats, $p < 0.001$, Figures 2 and 3). Removal of the endothelium prevented the Ach-induced vascular relaxation (Figure 2).

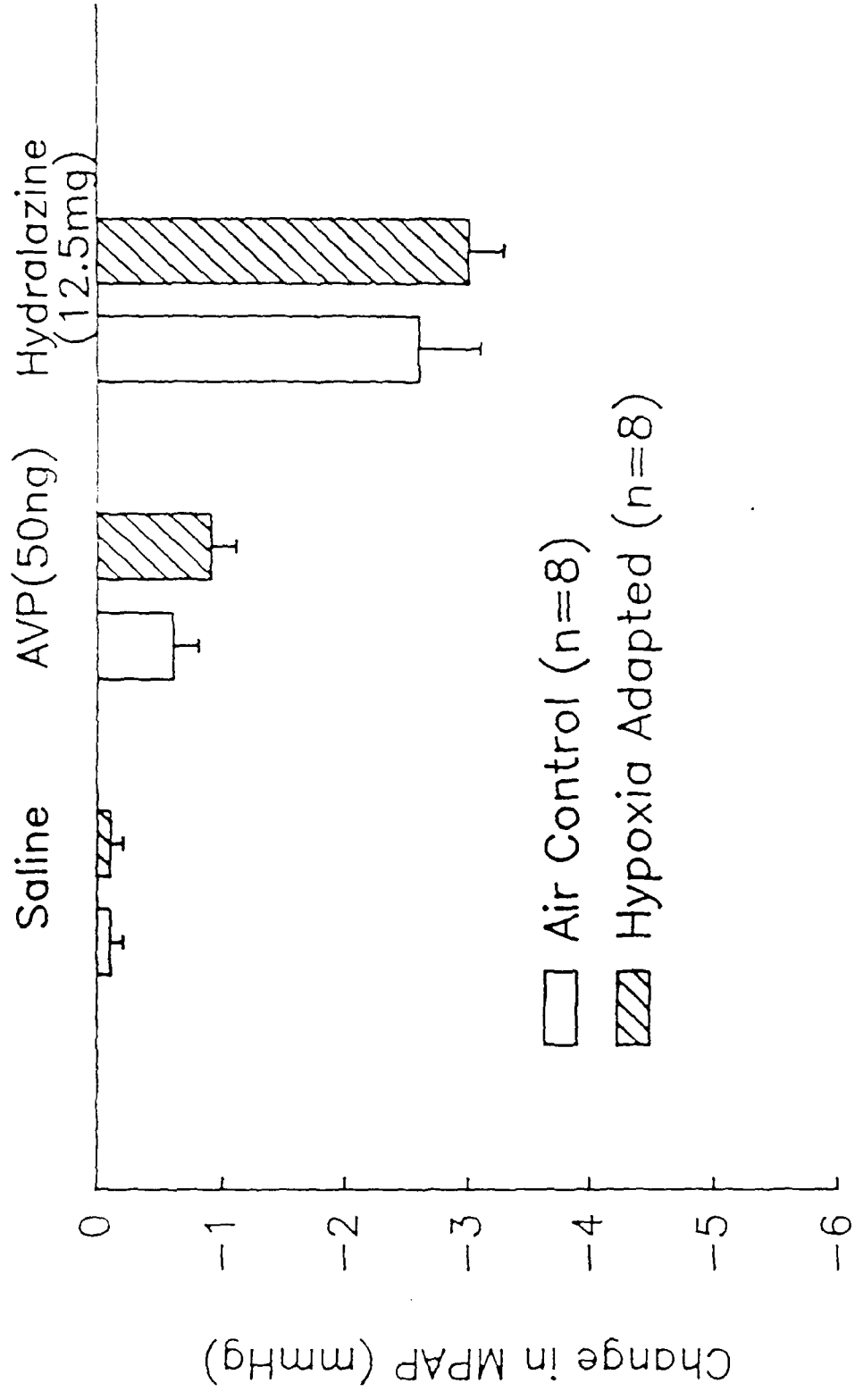
Thoracic aorta rings from hypoxia-adapted rats, with and without intact endothelium, were unable to maintain contraction to large doses of AVP; equivalently prepared rings from control rats performed significantly better (Figure 2, Table 2). The basis for the relaxation by rings from hypoxia-adapted rats to large doses of AVP cannot be determined from the current experiments. However, if the aortic ring responses to AVP were integrated over a restricted dose range of 10^{-10} to 3×10^{-8} M (a dose at which all

TABLE 1. Body Weight (BW), Wet-to-Dry Lung Weight Ratio and Basal Mean Pulmonary Arterial Pressure (MPAP) in the Isolated Perfused Lung Experiment

	BW (g)	MPAP (mmHg)	Wet/Dry Lung wt
Air control (n=8)	371.8±12.4	17.9±0.4	6.3±0.1
Hypoxia-adapted (n=8)	303.5±11.8**	33.2±0.6**	6.1±0.2

**p<0.01 compared to air control.

FIGURE 1: Jin et al. : Arginine Vasopressin lowers pulmonary artery pressure.



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FIGURE 2: Jin et al.: Arginine vasopressin lowers pulmonary artery pressure...

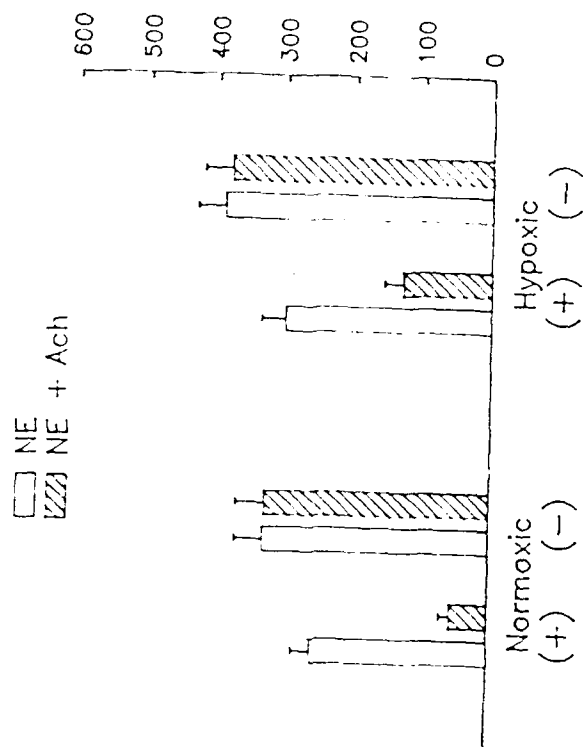
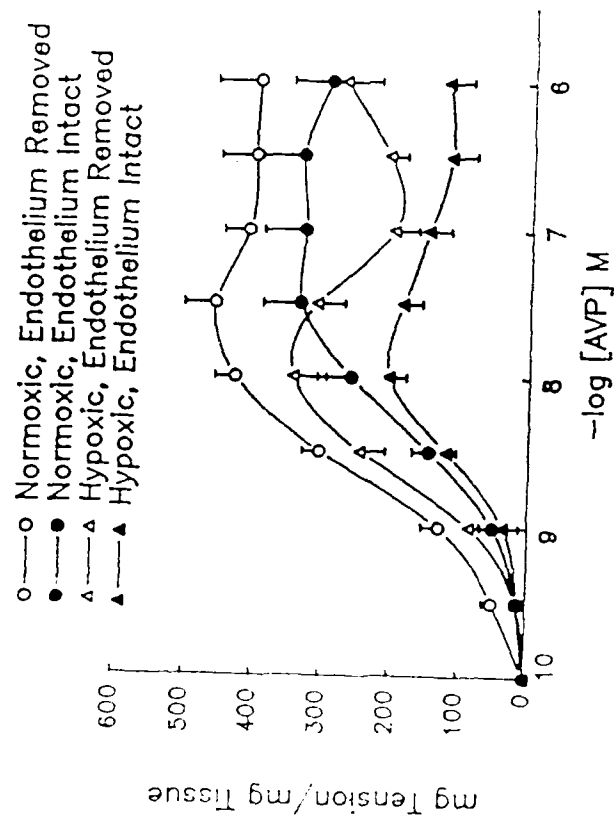


FIGURE 3: Jin et al.: Arginine vasopressin lowers pulmonary artery pressure.

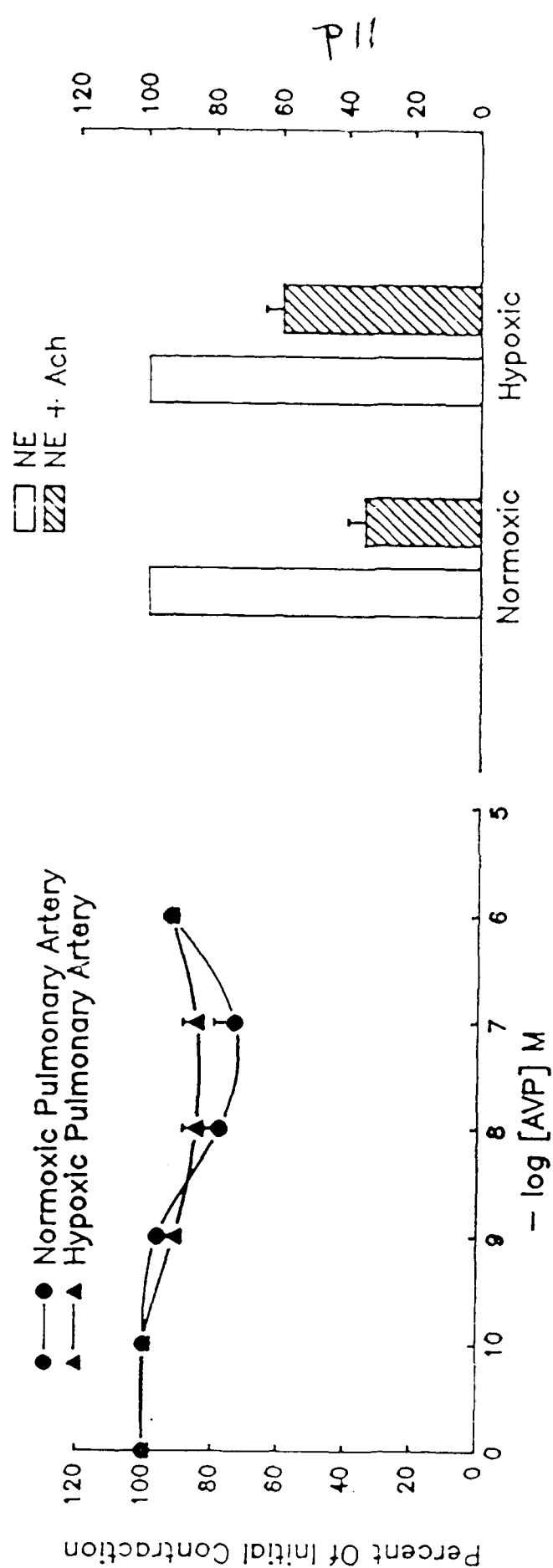


TABLE 2. Mean Integrated Dose and EC₅₀ Responses to AVP by Aortic Rings with Intact or Removed Endothelium From Normoxic and Hypoxia Treated Rats.

		N
Normoxic with intact endothelium		
10 ⁻¹⁰ to 3 X 10 ⁻⁸ M AVP	710±144*	9
10 ⁻¹⁰ to 10 ⁻⁶ M AVP	1808±330	9
EC ₅₀	8.43±.13**	8
Normoxic with removed endothelium		
10 ⁻¹⁰ to 3 X 10 ⁻⁸ AVP	1279±115 ^A	9
10 ⁻¹⁰ to 10 ⁻⁶ M AVP	2736±242 ^A	9
EC ₅₀	8.58±.14	9
Hypoxic with intact endothelium		
10 ⁻¹⁰ to 3 X 10 ⁻⁸ M AVP	521±.75	10
10 ⁻¹⁰ to 10 ⁻⁶ M AVP	1024±192 ^A	10
EC ₅₀	8.51±.07	8
Hypoxic with removed endothelium		
10 ⁻¹⁰ to 3 X 10 ⁻⁸ M AVP	952±140 ^B	10
10 ⁻¹⁰ to 10 ⁻⁶ M AVP	1893±260 ^{B,C}	10
EC ₅₀	8.63±.08	9

*Mean integrated dose response (mg tension/mg tissue vs. ln [AVP] M), mean ± SEM).

** -log [AVP] M, mean ± SEM

^A p<0.05 in comparison to Normoxic with intact endothelium.

^B p<0.05 in comparison to Hypoxic with intact endothelium.

^C p<0.05 in comparison to Normoxic with removed endothelium.

treatment groups had attained maximal contraction), then no significant differences in integrated dose responses (Table 2) between equivalently prepared rings isolated from hypoxia-adapted and control rats were apparent. Removal of the endothelium significantly enhanced contraction to AVP by aortic rings from both hypoxia-adapted and control rats; the improvement in performance could be attributed to an enhancement in the magnitude of contraction, since no increase in sensitivity (i.e. decrease in EC_{50}) to AVP was present in de-endothelialized aortic rings from either hypoxia-adapted or control rats (Figure 2, Table 2).

Pulmonary artery rings from hypoxia-adapted and control rats both manifested weak, but significant, vasodilatory responses to AVP. Rings isolated from hypoxia-adapted rats and pre-contracted by NE responded to AVP with increasing vasodilation; a dose of 10^{-7} M resulted a $14.6 \pm 0.2\%$ decrease from the initial NE-induced tension ($p < 0.01$, Figure 3). Similarly treated rings isolated from control rats exhibited a maximal decrease in tension of $28.2 \pm 0.4\%$ ($p < 0.01$, Figure 3); the magnitude of the vasodilatory responses of rings from hypoxia-adapted and control rats were not significantly different from each other. AVP was significantly less effective in inducing vasodilation in pulmonary artery rings pre-contracted by NE than was the endothelium-dependent mechanism activated by treatment with Ach. Rings isolated from hypoxia-adapted rats responded to Ach treatment with a $41.0 \pm 0.2\%$ relaxation in comparison to the $14.6 \pm 0.2\%$ relaxation measured after exposure to AVP ($p < 0.001$, Figure 3). Rings isolated from control rats exhibited a $65.1 \pm 0.2\%$ relaxation to NE versus a $28.2 \pm 0.4\%$ relaxation after treatment with AVP ($p < 0.001$, Figure 3).

There was no difference in the basal level of MSAP between the hypoxia-adapted and air control groups (Figure 4, Top panel). In contrast, the basal MPAP in hypoxia-adapted rats was significantly higher than in air controls (Figure 4, Bottom panel). Bolus injections of AVP (160 ng/kg) produced significant increases in MSAP in both air control and hypoxia-adapted rats. The systemic pressor response to AVP was blunted in the hypoxic group compared with the air control group. The AVP injection significantly lowered MPAP in hypoxia-adapted rats, but not in air control rats. Saline vehicle did not alter MSAP or MPAP in either group.

The basal plasma ANP concentration in hypoxia adapted rats (209 ± 33 pg/ml) was significantly higher than in air control rats (122 ± 22 pg/ml, $p < 0.05$) (Figure 5). Injection of AVP (160 ng/kg) produced a significant increase in plasma ANP in both hypoxia adapted and air control rats. Following AVP administration, plasma ANP concentration increased to 1346 ± 233 pg/ml, approximately 7 times pretreatment levels, in hypoxia adapted rats, and to 572 ± 174 pg/ml, approximately 5 times the basal level, in air control rats. The increase in plasma ANP following AVP injection was significantly greater in hypoxia adapted rats than in air controls. The interaction between the effects of AVP and hypoxia on plasma ANP levels was statistically significant by 2 way analysis of variance ($p < 0.05$). The AVP-induced increase in plasma ANP was completely abolished after injection of the AVP antagonist $d(CH_2)_5$ Try(Me)AVP in both hypoxic and normoxic groups (Figure 5), indicating that the AVP-induced release of ANP was V_1 receptor-mediated.

Injection of AVP did not change the ANP content of right atria in either hypoxia adapted or air control rats (Figure 6). Further, there was no significant difference in the pretreatment ANP content of the right atria

FIGURE 4: Jin et al: Arginine vasopressin lowers pulmonary artery pressure

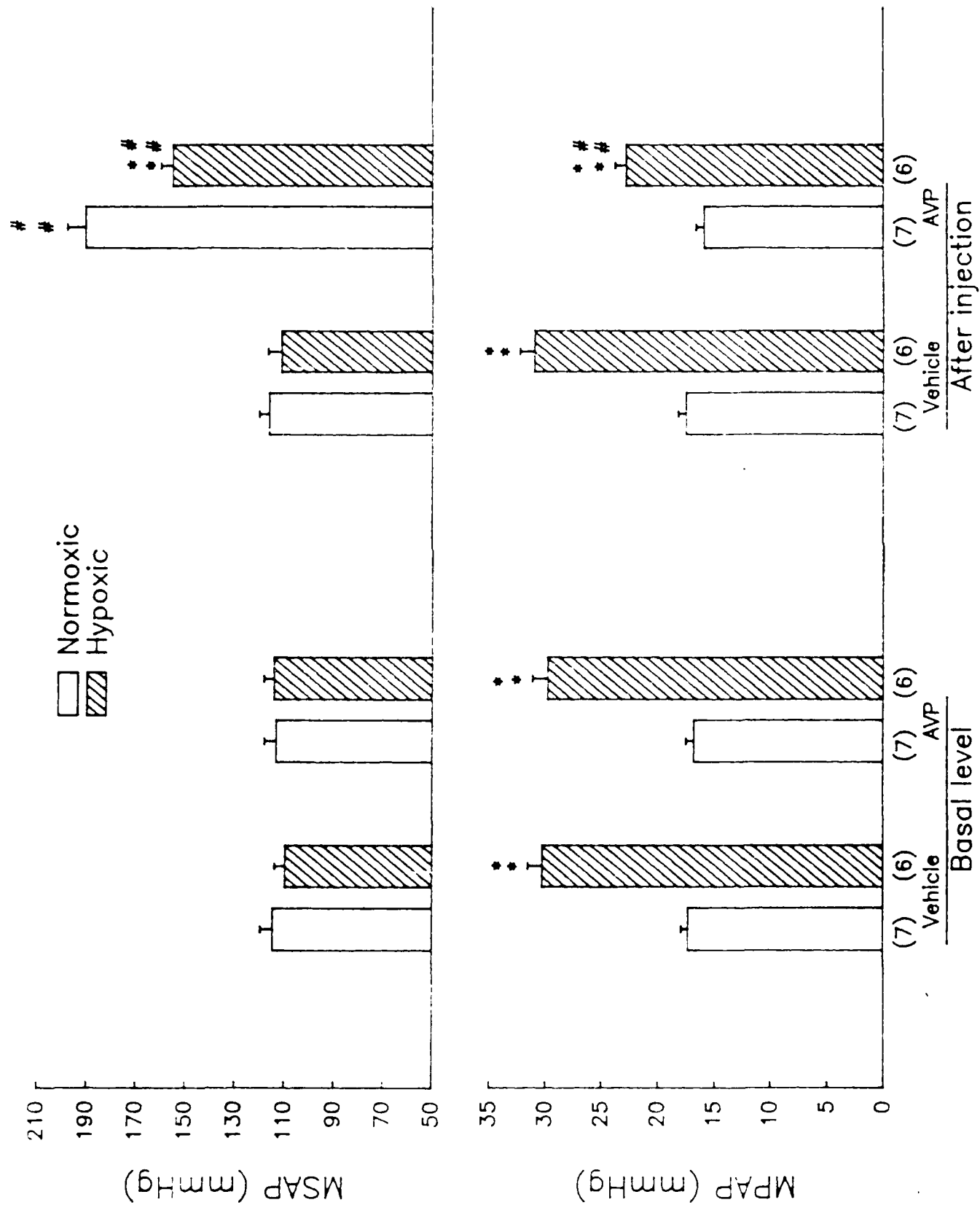


FIGURE 5: Jin et al.: Arginine vasopressin lowers pulmonary artery pressure.

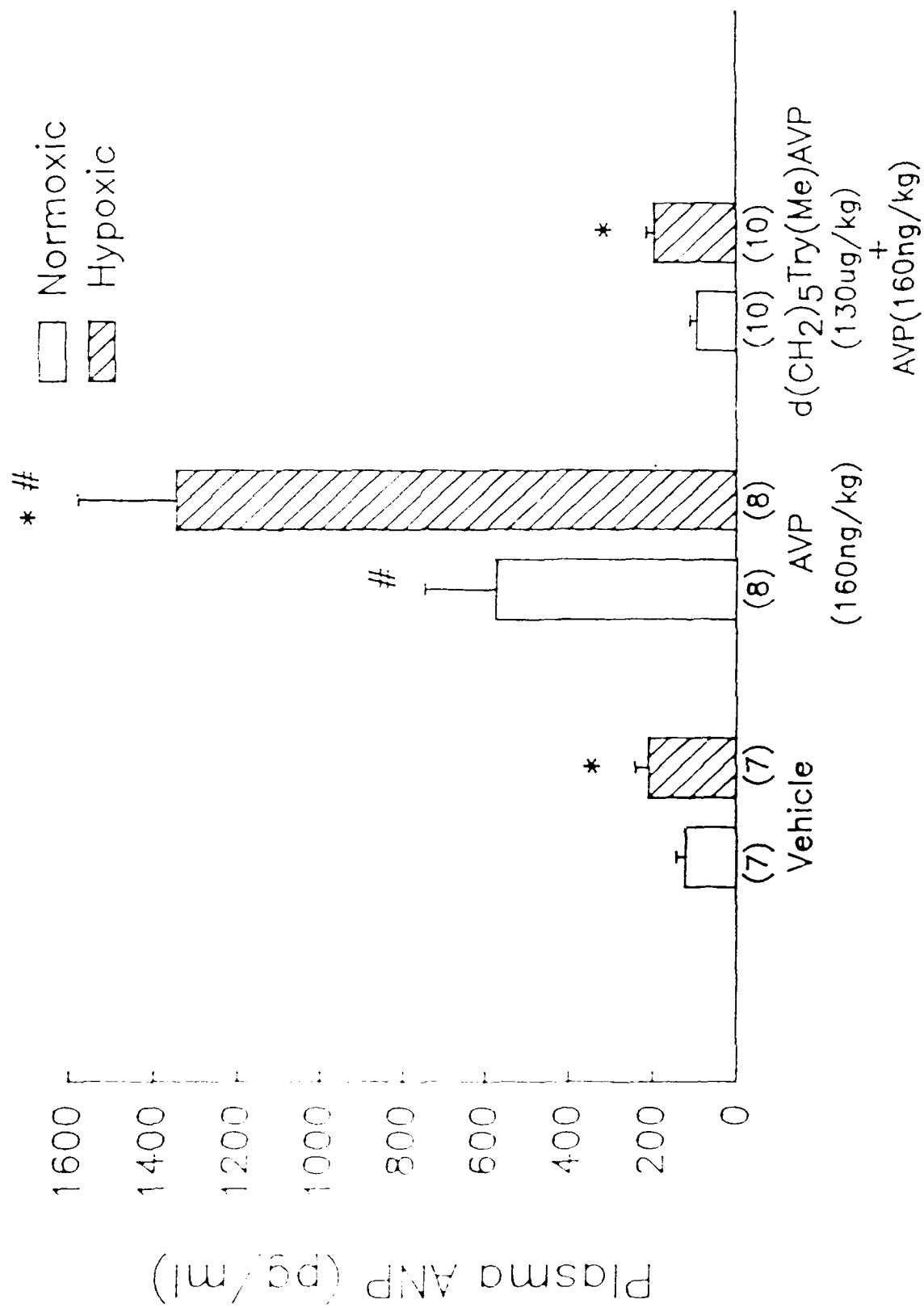
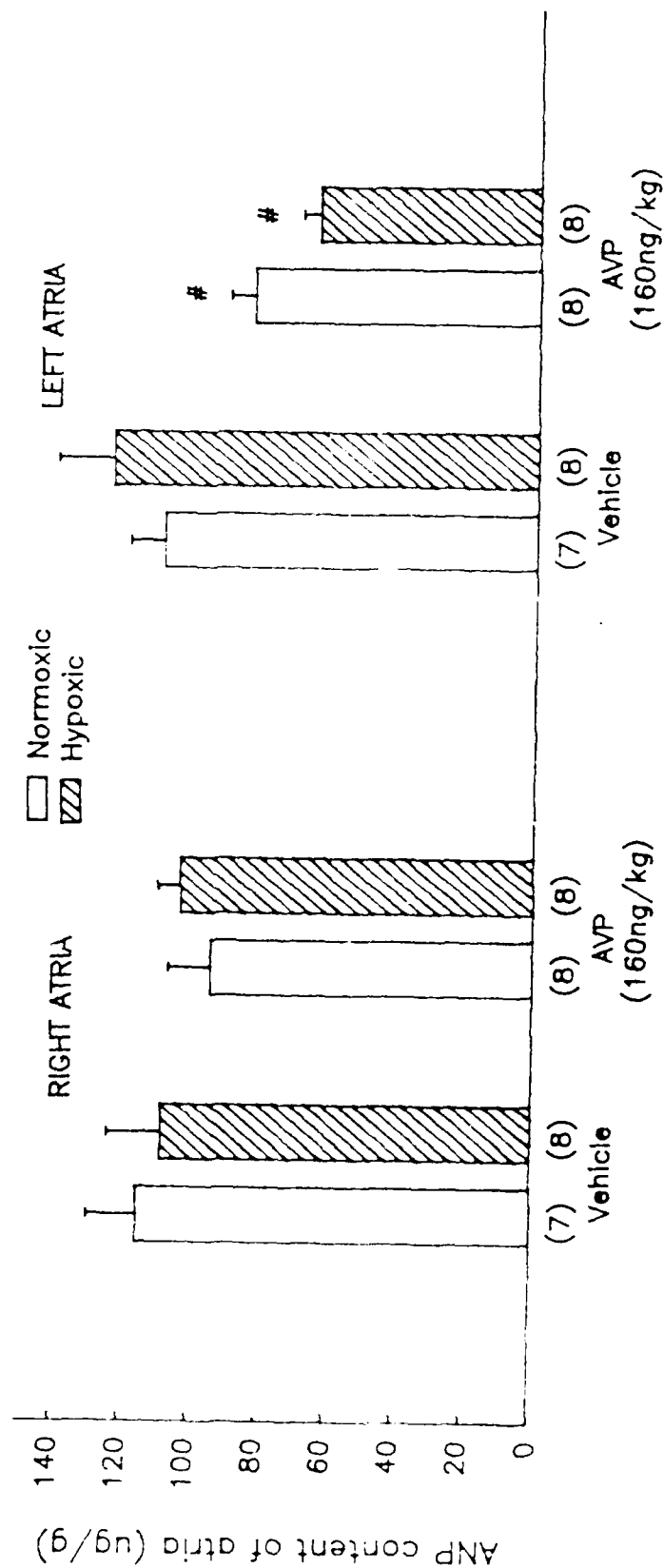


FIGURE 6: Jin et al.: Arginine vasopressin lowers pulmonary artery pressure



between normoxic and hypoxic groups. In contrast, AVP injection produced a significant decrease in the ANP content of left atria in both hypoxia adapted and air control rats (Figure 6). The decrement in ANP content of left atria was greater (approximately 50% of stores) in hypoxia adapted rats than in air controls (approximately 25% of stores), although the difference was not statistically significant. There was no difference in basal ANP content of left atria between the hypoxic and air control groups.

DISCUSSION

The current study confirmed our previous observation that administration of AVP produced a dose dependent pulmonary depressor effect in hypoxia-adapted rats, but had no significant effect on pulmonary artery pressure in air control animals (15,17). AVP had minimal vasodilator action in isolated, buffer perfused lungs and only a small vasodilator effect in pulmonary artery rings in both hypoxia-adapted and air control groups. These observations strongly suggest that the pulmonary depressor effect of AVP in intact hypoxia-adapted rats is not due to a direct action of AVP on the pulmonary vasculature. Published studies of the effects of AVP on the pulmonary circulation have yielded inconsistent results. Infusion of AVP has been shown to cause active, nonflow dependent pulmonary vasoconstriction in the intact conscious dog (26). Other in vivo studies have not consistently documented an effect of AVP on the pulmonary vasculature in rats, cats and human subjects (3,7,21,25,31,33). In the isolated perfused lung, AVP has been reported to increase pulmonary vascular resistance (28), yet most in vitro studies indicate that isolated pulmonary vascular smooth muscle is poorly responsive to AVP (4,26). Our current observations support the latter findings.

The current study further demonstrated that AVP induced exaggerated release of ANP from the left atrium into the circulation in hypoxia-adapted rats compared to air controls and that the AVP-induced release of ANP was completely abolished following pretreatment with the V_1 receptor antagonist in both hypoxic and normoxic groups. Taken together, our data suggest that the pulmonary depressor and blunted systemic pressor effects of AVP observed in hypoxia-adapted rats are related to augmented release of ANP from left atrium.

Pretreatment with a V_1 receptor antagonist nearly abolished the pulmonary depressor effect of AVP in hypoxia-adapted rats, whereas a V_2 receptor antagonist had no effect on the AVP induced response (17), indicating that the depressor effect of AVP in the pulmonary circulation in hypoxia-adapted rats is V_1 receptor mediated. We have considered several mechanisms by which V_1 receptors may mediate the pulmonary depressor effect of AVP in hypoxia-adapted rats. One possible explanation is that V_1 receptors, which are absent from the pulmonary vasculature under normal conditions, may appear de novo in the pulmonary circulation of hypoxia-adapted rats. Alternatively, the second messenger mechanism through which AVP operates may be altered in hypoxia-adapted pulmonary vessels. Hypoxia causes neomuscularization of the pulmonary vasculature (29), and the response of these new smooth muscle cells to AVP may differ from that of normal pulmonary vascular smooth muscle. The first explanation seems unlikely, since circulating AVP levels are increased significantly in animals (10,30,32) and humans (1,6,12,13,34) adapted to acute and chronic hypoxia, thus favoring down regulation of V_1 receptors. Further, the observations in the current study that AVP had no significant direct vasodilator or relaxant action in either the isolated, non-blood perfused lung or pulmonary artery rings from either hypoxia adapted or air control rats, rule against either explanation.

Exogenous AVP decreases cardiac output in conscious rats (27,39) and dogs (22,26,28), providing another potential mechanism for the pulmonary depressor effect of AVP in hypoxia-adapted animals. We have previously demonstrated that the magnitude of the AVP-induced reduction in cardiac output in hypoxia adapted rats is not different from that in air controls (17), suggesting that the pulmonary depressor response to AVP in hypoxia adapted rats is not

secondary to the fall in cardiac output. Calculated pulmonary vascular resistance increased significantly after AVP injection in air controls, but fell slightly in hypoxia-adapted rats, suggesting that the failure of the pulmonary vascular resistance to increase following AVP administration in hypoxia-adapted rats may have accounted for the AVP-induced fall in pulmonary artery pressure. A likely explanation for this observation is that AVP induces the release of compounds that can dilate the pulmonary vasculature, such as ANP.

The current study tested the hypothesis that enhanced AVP-induced ANP release accounts for the pulmonary depressor response and blunted systemic pressor response to AVP seen in hypoxia adapted rats. AVP injection produced a significant increase in plasma ANP and a significant decrease in left atrial ANP in both hypoxia adapted and air control rats. The post injection increase in plasma ANP and decrease in left atrial ANP were significantly greater in hypoxia adapted rats than in air controls. Further, the AVP-induced increase in plasma ANP was completely inhibited by pretreatment with the V_1 receptor antagonist ($d(CH_2)_5$ Try(Me)AVP, 130 ug/kg, i.v.) in both hypoxia adapted and air control rats. This dose of the V_1 receptor antagonist abolished completely the pulmonary depressor and systemic pressor effects of AVP in hypoxia adapted rats (15). Recent studies have shown that ANP relaxes pulmonary artery segments in vitro (5,23). Our previous studies also demonstrate that ANP produces a dose dependent reduction MPAP which is significantly greater in hypoxia adapted than in air control rats, and that the enhanced pulmonary depressor effect of ANP in hypoxia adapted rats is due mainly to a direct vasodilator effect on the pulmonary vasculature (16). Taken together, the data support our hypothesis that augmented AVP-induced

release on ANP accounts for the pulmonary depressor effect and the blunted systemic pressor effect of AVP observed in hypoxia adapted rats.

The mechanisms by which AVP induces release of ANP are unclear. Other AVP analogs, such as dAVP (1-deamino-Arg⁸-vasopressin) and oxytocin, which have pressor activity, and several other pressor agents, such as phenylephrine and angiotensin II, induce ANP release in the same dose-dependent manner as AVP in intact anesthetized rats (19). In contrast, neither the nonpressor analog dDAP nor AVP in the presence of its antipressor (V_1) antagonist causes ANP release. These observations suggest that AVP-induced ANP release is related to the systemic pressor action of AVP. The finding from our current study that AVP induced ANP release mainly from left atria is consistent with the above interpretation. The AVP-induced increase in systemic pressure is not the only factor involved in ANP AVP-induced release of ANP, however. Manning et al. (19) have found that, although acute increases in systemic blood pressure were maximal after the administration of 1 ug of dAVP, ANP release continued to increase at doses up to 10 ug (doses of dAVP in excess of 10 ug were not tested). Further, there were significant differences in the amount of ANP released in response to matched increases in MSAP within 15 min after injection of AVP, dAVP, phenylephrine and angiotensin II. In the current study, a blunted systemic response to AVP was associated with augmented ANP release in hypoxia adapted rats compared to air controls, suggesting the possibility that factors other than increased blood pressure and atrial stretch are involved in the AVP-induced release of ANP.

Hypoxia per se stimulates release of ANP from atrial stores. Perfusion of isolated rat and rabbit hearts with hypoxic ($PO_2 = 21 \pm 4$ mmHg) buffer via a Langendorff apparatus for 10 min periods has been associated with

approximately 4-fold increases in ANP levels in the cardiac effluent (2). Further, rats exposed to chronic hypobaric hypoxia (0.5 atm X 21 days) developed significant increases in plasma ANP levels (baseline 101 ± 10 pg/ml; 21 day hypoxia 238 ± 107 pg/ml, $p < 0.05$) (20). Our finding of significantly higher plasma ANP levels in hypoxia-adapted rats compared to air controls confirms this finding.

One possible explanation for the blunted systemic pressor response to AVP in hypoxia adapted rats, compared to air controls, is that V_1 receptors are downregulated in the systemic vasculature of hypoxia adapted rats. The observation that circulating AVP levels are increased significantly in acute and chronic hypoxia (1,6,10,12,13,30,32,34) is consistent with this interpretation. However, the current study demonstrated no significant difference in contractile response to AVP at a dose range of 10^{-10} to 3×10^{-8} M of aortic rings from hypoxia adapted vs. air control rats, thus failing to support this interpretation. Another possibility is that AVP may stimulate vasodilator V_2 receptors in the systemic circulation that may be upregulated in hypoxia adapted animals. Our previous experiments (17), in which rats were pretreated with the V_2 receptor antagonist (2-Ile-4-Val-AVP) prior to AVP administrations, failed to support this hypothesis. A third possible explanation for the blunted systemic pressor response is that AVP may cause an exaggerated fall in cardiac output in hypoxia adapted rats. Our previous

experiments (17) showed similar percent decreases in cardiac output in hypoxia adapted and air control groups, thus failing to support this interpretation. Finally, AVP may blunt its own V_1 receptor mediated systemic pressor response by stimulating the release of systemic vasodilators, such as ANP, in hypoxia adapted rats. Our current experiments support the hypothesis that the blunted systemic pressor effects of AVP observed in hypoxia adapted rats may be related to augmented release of ANP.

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FIGURE LEGENDS

- Figure 1: Effects of AVP and hydralazine on mean pulmonary arterial pressure (MPAP) in isolated perfused lungs from hypoxia adapted and air control rats. Data are presented as means \pm SEM.
- Figure 2: Contractile responses to cumulative doses of AVP (left) and relaxation to Ach (right), by thoracic aorta rings isolated from hypoxia-adapted and control rats. Rings possessed an intact endothelium (+), or were de-endothelialized (-). Open histograms (right) denote maximal contractile response to 3×10^{-7} M NE, striated histograms denote remaining contraction subsequent to application of 10^{-6} M Ach. All data are presented as mean \pm SEM, N=9 to 10 per treatment group.
- Figure 3: Vasodilatory responses to cumulative doses of AVP (left) and 10^{-6} M Ach (right) by endothelium-intact pulmonary artery rings isolated from hypoxia-adapted and control rats. Vasodilatory responses to both agonists are presented as the percentage remaining of an initial contraction induced by 3×10^{-7} M NE. All data are presented as mean \pm SEM, N=9 to 10 per treatment group.

Figure 4: Effects of AVP (160 ng/kg) on mean systemic arterial pressure (MSAP) and mean pulmonary arterial pressure (MPAP) in hypoxia-adapted and air control rats. Data are presented as mean \pm SEM.
* $p < 0.01$, compared to the respective normoxic group.

$p < 0.01$, compared to the respective basal or vehicle group.

Figure 5: Effects of AVP (160 ng/kg) on plasma ANP levels in hypoxia-adapted and air control rats. Data are presented as mean \pm SEM.
* $p < 0.05$ compared to the respective normoxic group.
$p < 0.05$ compared to the respective vehicle or $d(CH_2)_5$ Try(Me)AVP + AVP group. Numbers in parentheses = number of rats in each group.

Figure 6: Effect of AVP (160 ng/kg) on ANP content of right and left atria in hypoxia-adapted and air control rats. Data are presented as mean \pm SEM.
$p < 0.05$ compared to the respective vehicle.
Numbers in parentheses = number of rats in each group.